HERPETOMONAS PEDICULI, Nov. Spec., PARASITIC IN THE ALIMENTARY TRACT OF *PEDICULUS VESTIMENTI*, THE HUMAN BODY LOUSE*

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Plate I

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INTRODUCTION

The organism which forms the subject of this paper is a small flagellate Protozoön occurring in the alimentary tract of the body louse, *Pediculus vestimenti* (*P. corporis*). The flagellate belongs to the genus Herpetomonas, and is, I believe, now recorded for the first time. I propose for it the name *Herpetomonas pediculi*, using the name Herpetomonas in the sense of Saville-Kent (1881), the founder of the genus.

The parasite was first seen by me nearly three years ago, when working in Cambridge. At that time, material being scanty and

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apparently difficult to obtain, there seemed to be no special reason for recording the presence of yet another species of Herpetomonas, though the possibility of lice acting as carriers of disease was realised. Other researches were then in progress, but I have given *H. pediculi* intermittent attention ever since I discovered it. Lately it has been suggested that flagellates belonging to the genera Herpetomonas and Crithidia, occurring in the digestive tracts of blood-sucking insects, are really stages in the life-history of vertebrate trypanosomes. In consequence of the importance of the subject in relation to the transmission of trypanosomes, and of the increasing attention devoted to leishmaniasis (or human herpetomoniasis), I have resumed my study of *Herpetomonas pediculi*, and have found its complete life-cycle.

I hope to show that, although *Herpetomonas pediculi* might easily be confused with dangerous parasites transferable to man by the bite of the insect hosts, yet it is really a harmless flagellate of the gut of the louse. Bred lice, fed on my own blood, have been used in the research.

MATERIAL AND METHODS

The hosts of *Herpetomonas pediculi* were common body lice, *Pediculus vestimenti*. The lice were originally obtained from the bodies of infested children, and from verminous clothing recently removed from the body. Some of the lice so obtained were dissected, and found to contain Herpetomonads. The adult lice were isolated into glass tubes, containing small fragments of white woollen material. They clung to the material, and excrement from them adhered to it. Examination of the said excrement in a very few cases showed non-flagellate stages of the parasite. Lice, thus known to be infected, were then paired, as also were lice that were apparently uninfected. The parent lice were kept in closed tubes in contact with my body, and were fed twice daily, either on my arm or on the back of my hand. They fed greedily, often evacuating faeces as they fed, and at times even unaltered fresh blood. The eggs from these lice were kept also in tubes, and, when the larvae hatched, they were treated in the same way as the adults.

Detailed examination was made of the eggs, larvae, and adult

lice, and it was found that bred lice from parents known to be infected were parasitised by *H. pediculi.* Though clean lice bred clean, the young ones became infected when they came in contact with the faeces of infected lice.

Again, faeces known to be infected were smeared on spots at which uninfected young lice were allowed to feed. Three days later, control insects showed no flagellates or other form of the parasite, while those fed at infected spots contained active flagellates when dissected.

The sole food supply of the bred lice was my own blood. Cultures of my blood on Mathis' modification of Novy and MacNeal's medium have never yielded any flagellates of any sort.

The percentage of infected lice is not a high one—I have dissected as many as fifty lice at a time without finding Herpetomonads—nor can the infection be described as heavy; three hundred Pediculi were examined, of which twenty-five were found to be infected. Much time has been devoted to the examination of fresh material, which is absolutely essential if the life-cycle is to be followed with exactitude. The gut of the louse was divided into small serial portions, each of which was finely teased and examined in physiological salt solution. Observation of the living organisms was supplemented by examination of thin smears of the gut contents, teased intestinal wall, and other organs, and long search was made for possible intracellular stages.

Wet fixation with osmic vapour, followed by absolute alcohol, was used, and coloration with Giemsa's or other modification of the Romanowsky stain. Very dilute solution of methylene blue was of use as an *intra vitam* stain.

It may be noted that lice dissected when several hours had elapsed since the last feed showed the parasites better than recently-fed ones. The presence of much blood in the gut interferes with the study of the living organism, as well as adds to the difficulty of staining.

OCCURRENCE OF THE PARASITES IN THE HOST

Herpetomonas pediculi exhibits three typical stages in its life-history—the pre-flagellate, flagellate, and post-flagellate stages. So far the examination of the mouth parts of the lice has shown no

parasites. The pre-flagellate stage of the parasite occurs chiefly in the oesophagus and proventriculus of the adult louse, and throughout the alimentary canal of the immature insect or larva. The flagellate stage is found at its best in the mid-gut of the mature louse, while the extreme intestinal and rectal portions of the gut contain the post-flagellates. The latter are also found in the excrement. The late larva may contain a few flagellate parasites.

Detailed examination was made of the reproductive organs and ova of the lice, but no parasites were found therein. The possibility of the hereditary infection of the lice with *H. pediculi* was, in my opinion, excluded so far as the specimens that I examined were concerned. The parasites have not been found in organs other than those of the alimentary tract. The diverticula of the alimentary canal apparently are uninfected.

MOVEMENTS

Movements of *H. pediculi* are most easily observed in the fully developed flagellate stage. They are also very vigorous during division, but as the object of the said movements then is to complete the fission of the organism, they are of a somewhat exceptional character.

Usually movement is brought about by waves of contraction followed by relaxations passing down the body, while progression is aided by the lashing of the flagellum, which is forwardly directed. The type of movement is somewhat euglenoid, and the occasional concentration of the cytoplasm into the anterior end produces a 'peg-top' effect that is very characteristic. The progression is somewhat spasmodic, consisting of alternate slow movements and rapid darts forward, accompanied by a slight rolling from side to side. Reversal of the direction of motion is easily accomplished. The organism either swings as a whole in a semicircle, the posterior end acting as a centre of rotation, or the flagellum bends back parallel to the body, which then swings suddenly in a semicircle, and so comes to lie in a straight line with the flagellum, after which the parasite moves away in the opposite direction from what it was traversing previously.

The flagellum often lashes vigorously, and when obstacles are

encountered appears to test them by touching them rapidly in different spots.

Rotatory movements occur when the Herpetomonas becomes fixed to *débris* in the lumen of the gut. The organism lashes its flagellum violently, and the flagellum describes a series of circles and spirals, producing marked currents in its neighbourhood.

A very common movement is that of partial rotation of the body of *H. pediculi*. The posterior end of the body remote from the nucleus is chiefly concerned, and this portion often twists over, so that the dorsal surface becomes ventral, and *vice versâ* (Plate I, fig. 10). Sometimes a small cluster of parasites may be observed, twisting simultaneously and producing a general shimmering effect.

Entanglement of two organisms by their flagella is sometimes seen. The movements of the parasites then are most violent. On one occasion I saw the flagellum of a small parasite torn from its body by the vigorous movements of a much larger Herpetomonas with which it had become entangled. Such intensely vigorous movements as the last-mentioned are rare.

LIFE HISTORY IN BRIEF OF *HERPETOMONAS PEDICULI*

It is of interest to trace the course of the development of the parasite throughout its life in one host. The following development has been observed in the living organism, and afterwards corroborated by examination of stained preparations.

In the excrement of lice infected with *H. pediculi* are small, oval bodies, well adapted for resisting desiccation or other adverse condition. Similar bodies occur in the hind gut of the lice, where they are formed before passing out with the excrement. Other lice, feeding at spots contaminated by their predecessors, ingest some of these small oval post-flagellates, which consist of a 'varnish-like' thin cyst wall, enclosing some granular protoplasm, a nucleus and a blepharoplast (Plate I, figs. 27-29). Such cysts may also be ingested by larval Pediculi. Passing with fresh blood into the fore gut of a new host, the post-flagellates commence a new development, and as this leads to the formation of the flagellate, it has been termed the pre-flagellate stage (fig. 1).

At first round or oval, the parasite rapidly commences to

elongate, the end first lengthening becoming, as a rule, the flagellar end of the organism. At this period, a somewhat more refractile area can be seen in life, and the finely granular chromatophile contents of this area concentrate, forming a thread which ultimately reaches the surface. The thin ectoplasm of the parasite is pushed forward still more by the thread, which ultimately becomes free of the body and protrudes as a short flagellum (fig. 2). The flagellar origin is the chromatophile area, or so-called ' flagellar sac,' which is usually in the neighbourhood of the blepharoplast, which, in the fully developed organism, is always anterior to the nucleus. Growth of the non-flagellar end is continuous, and by the time that the flagellum has reached its full length, the non-flagellar (or posterior) end has elongated and become fully developed.

During the actual flagellate stage (figs. 8-18) little in the way of actual development occurs, but the vital activities of the organism are displayed by vigorous multiplication. Increase in numbers in *H. pediculi* takes place by longitudinal division. The parasites about to divide seem to grow broader just prior to the act, and to become more granular. The first indication of division is shown by the concentration of the substance of the blepharoplast into two masses, which are connected together by a very narrow neck. The dumbbell-like body so formed (fig. 19) gradually separates into two distinct blepharoplasts, placed slightly obliquely, one on either side of the body (fig. 20). The intra-cellular part of the flagellum (or rhizoplast) commences to divide just after the blepharoplast, and the split appears to extend forwards. Concentration of the nuclear material occurs simultaneously, and the nucleus becomes constricted, usually in the median line and parallel to the long axis of the body, but occasionally markedly to one side. The constriction deepens, and ultimately two nuclei are produced. These migrate to the sides of the organism, and fission of the general cytoplasm commences (figs. 20, 21). The flagella lash about very much at this time, and their vigorous action aids in the separation of the daughter organisms. The latter gradually diverge until they come to lie in a straight line, and finally become separated from one another at the apex. The fission is practically always followed by active swimming movements of the daughter organisms. Consequent on this great activity of the daughter forms immediately after division,

rosettes of Herpetomonads, due to repeated longitudinal division and non-separation of the resultant parasites, are exceptional. Division is best seen in the mid-gut of the louse.

After a series of longitudinal divisions, resulting in the production of a number of flagellate individuals, a reaction sets in, and the parasite prepares for life outside the body of its host. As the Herpetomonad passes backwards into the very dark semi-digested blood in the hind gut of the louse, the chromatin of its flagellum dwindles, and appears to be absorbed (figs. 22-25). The cytoplasm concentrates around the nucleus, to which the blepharoplast also is drawn nearer. The parasite becomes more or less rounded or oval, and proceeds to secrete a thin, gelatinous wall, which rapidly hardens to a 'skin-tight' coat around the organism, which, in this sense, may be said to encyst (figs. 26-28). Thus prepared and protected, the oval bodies, now known as post-flagellates, pass from the gut of the host, mingled with the dejecta, to recommence the life-cycle if ingested by a new host.

MORPHOLOGY

A. The Pre-flagellate Stage

The pre-flagellate stage (figs. 1-4) of *H. pediculi*, at its earliest, takes the form of small oval or rounded bodies, measuring 6μ to 7μ by 4μ to 5μ. They have a marked resemblance to the Leishman-Donovan bodies. The pre-flagellate shows a thin ectoplasm and endoplasm containing refractile granules. The nucleus (fig. 1) is oval, or occasionally rounded, while the deeply staining blepharoplast (kinetic nucleus) may be bar-like, oval, or occasionally rounded, and is well marked (figs. 1, 2). The position of the blepharoplast varies somewhat, as would be expected in a developing organism. It may lie to the side of the nucleus or above it, and occasionally the blepharoplast is apposed to the nucleus. The chromatophile area (fig. 1) from which the flagellum differentiates is also present. Division occurs in the pre-flagellate stage (fig. 5), more especially when the organism is beginning to elongate, and possesses a short flagellum. Isolated chromatoid granules are sometimes present in the pre-flagellates (fig. 2). The appearance of inter-

mediate forms (figs. 2-4, 6, 7) between the pre-flagellate and the flagellate has been indicated in the section dealing with the life-history.

B. The Flagellate Stage

The flagellate form (figs. 8-18) of *H. pediculi* is an active organism relatively small compared with other Herpetomonads, its body length varying from 11μ to 26μ in the specimens examined. The inclusion of the flagellum doubles the length of the organism, for the flagellum itself may be 30μ in length (fig. 16). The cytoplasm of the organism is finely alveolar (figs. 8-18), and very refractile in life. Chromatoid granules (figs. 15, 18) are present in some cases. The protoplasm rarely presents marked vacuoles in stained preparations, but in life a clearer area is sometimes seen near the origin of the flagellum.

The nucleus (figs. 8-18) is round or oval, and crowded with very fine granules. A karyosome is seen in some cases (figs. 11, 15), but is not visible in all, doubtless being masked by the numerous fine granulations present. A nuclear membrane apparently occurs, but is not so chromatic as when the nucleus is of a vesicular type. The blepharoplast (kinetic nucleus) may be oval or rod-like, lying transversely across the body, or somewhat obliquely. Occasionally it is curved, and it often presents a bowed appearance prior to division. It stains deeply, taking a purplish tint with Giemsa. Before the onset of the multiplicative phase the blepharoplast presents no differentiation. The free flagellum tapers finely at its free end. It originates as a rhizoplast near the blepharoplast, and occasionally a minute basal granule can be distinguished with difficulty.

Aggregation rosettes. Just as division rosettes are infrequent, so aggregation rosettes, or clusters, are uncommon. It is noteworthy that the members of an aggregation rosette (fig. 13) may be of different sizes and ages. In such rosettes, the parasites either mass themselves around some food particle with which they are in contact by their flagella, or else several organisms intertwine their flagella and so form a sort of bouquet or ball of living organisms, all vibrating slowly from a common centre provided by their interlaced

flagella. These rosettes in time break up into the component units. One after another, the slow-moving organisms manage to detach themselves and swim away until the last two separate. The object underlying these simple rosette formations is not fully understood. Possibly it enables the flagellates to withstand better any currents in the gut, and so gives them a somewhat longer lease of life as flagellates, before encystment overtakes them.

C. The Post-flagellate Stage

The post-flagellate stages of *H. pediculi* (figs. 25-29) when fully formed are small bodies (less than the pre-flagellates) containing protoplasm and a nucleus, to which the blepharoplast may be apposed, or in which nucleus and blepharoplast can be distinguished as separate entities. The blepharoplast is often somewhat smaller than in the other phases of the parasite. The cyst wall is extremely thin, staining pinkish after Giemsa (figs. 27, 28). The blepharoplast is not always easy to demonstrate in stained preparations, but that it must be present is obvious when one has taken the trouble to watch the process of post-flagellate formation in the living animal, and has studied a series of stained preparations of intermediate forms (figs. 22-26). Cysts with thick, radially-striated walls (fig. 29) have very rarely been encountered, and I am inclined to think that the presence of the swollen gelatinous wall containing striations, and enclosing a parasite with chromatoid granules, is a sign of degeneration.

HERPETOMONAS PEDICULI IS A NATURAL PARASITE OF *PEDICULUS VESTIMENTI*

Recently much controversy has arisen from statements made to the effect that flagellates found in sanguivorous insects must be regarded as developmental stages of trypanosomes. Accordingly—ignoring the evidence of life-cycle and morphology—*H. pediculi* would be regarded by some as a phase of a trypanosome. Sweeping statements such as that quoted are rarely logical, and when they are based upon a series of speculations and single instances, instead of on an accumulation of facts, they are usually unsound.

While it is quite true that certain trypanosomes, e.g., *T. lewisi*, assume a Herpetomonas-like form in cultures, yet they are not then under exactly natural conditions. Further, they may be considered as reverting to the type from which it seems probable that they have originated, namely, primitive Herpetomonads which have undergone morphological changes and in process of time have evolved the trypanosome type when inoculated into the vertebrate host.

With regard to *H. pediculi*, I do not think that there is any doubt that it is a flagellate, natural to and parasitic in the insect host, and that it has no connection with a human trypanosome, pathogenic or non-pathogenic. In support of this conclusion, I cite the following facts and experiments:—

(1) At various times during the past three years I have fed lice on my blood from the time of hatching until they died. A tsetse fly transmitting *Trypanosoma gambiense* is at first limited in its period of infective inoculation. Lice might also be similarly limited, but, owing to the method of feeding adopted, no question of the lice not having fed at their infective period can be entertained. In spite of repeated feedings of lice, my blood shows no signs of trypanosomes, whether tested by ordinary microscopical examination of films, by thick films, or by cultural methods, and the period covered by the experiments is ample to have allowed of full development of trypanosomes, were *H. pediculi* a phase of one.

(2) Artificially infected lice have been fed simultaneously. The result of mass feeding surely should have been sufficient to produce some indication of trypanosomes, were any present. No such indications have been found, even after inoculation of my blood into susceptible animals like white rats. (Animals examined for six weeks after inoculation.)

(3) The experiment of inoculating rats with the contents of the gut of lice containing *H. pediculi* has given no positive results whatever. The rodents remained perfectly healthy, nor did cultures of their blood, or thick film examinations, yield any trace of trypanosomes.

(4) Cultures of the gut contents of infected lice showed no further stage in the life-history of the parasite.

(5) The methods of infecting larvae and adults of *P. vestimenti* with *H. pediculi* have been briefly indicated in a preceding section.

The same contaminative method of infection has been observed under natural conditions, and resembles that found in the case of some other insects, such as *Pulex irritans* (adult and larva), infected with *Crithidia pulicis* (Porter, 1911) and *Nepa cinerea*, harbouring *Herpetomonas jaculum* (Léger, 1902; Porter, 1909).

Further, the well defined development of *H. pediculi*, with its pre-flagellate, flagellate and post-flagellate forms, presents a cycle complete in itself, and there is no evidence to show that there is any connection with the life-cycle of any other organism.

Contamination of experimental *P. vestimenti* by feeding on other vertebrates has been rigorously excluded, so that no fallacious results can accrue from outside sources.

From the foregoing considerations, the conclusion obviously must be that if *H. pediculi* be a stage in the life-history of a vertebrate trypanosome, the said trypanosome should most probably be present in my blood, and should have revealed itself by now. Repeated cultures, thick-film blood examinations, and ordinary smears, examined continuously during this research, have all proved negative. Hence, all the evidence available points to the fact that *H. pediculi* is a parasite of the insect *Pediculus vestimenti*, and has no connection with any trypanosome of persons on whom lice may feed. Were such a trypanosome to exist, it is surprising that it has not been recorded ere this, considering the number of blood examinations undertaken in various scientific institutions.

Further, I do not think that *H. pediculi* has any connection with *Leishmania*, as no symptoms of leishmaniasis have developed in me, and England is a country free from the disease. However, the possible occurrence of such a natural *Herpetomonas* in lice must be remembered in experimenting with Pediculi as possible transmitters of *Leishmania*.

'Wild' lice—the term commonly used to denote lice that were not bred for purposes of investigation, but collected at random—obtained from several widely different districts in England, have also yielded the flagellates when dissected. Doubtless, were more lice available from other areas, some also would be infected. The inference is then, I think, fairly justified that *H. pediculi* occurs in a few body lice throughout England.

Some Continental authorities would, perhaps, place *H. pediculi*

in the genus *Leptomonas*. However, I have followed most English workers in considering that members of the genus *Herpetomonas* are really uniflagellate, as originally defined.

NOTE ON THE BIOLOGY AND LIFE-HISTORY OF *PEDICULUS VESTIMENTI*

The study of parasitic Protozoa demands a good knowledge of the life-history and habits of the host. In dealing with lice, great difficulty was at first experienced, as the literature on the subject is very scattered and unsatisfactory. Since the commencement of this research, a valuable paper by Warburton (1909) has appeared, which gives details as to the length of life of the lice, time of incubation, and rearing of the larvae. I can fully confirm all that Warburton has recorded.

The eggs of *P. vestimenti* vary in their incubation period. I found that while a few eggs hatched in four to five days, others matured as much as six weeks after laying. Warburton found the same kind of variation. The larvae were pale coloured, and fed as soon as they left the egg, if placed on the back of the hand. Moulting occurred every four days, the new skins being slightly darker than the previous ones. The larvae fed very greedily, and were much more active than the adults. When feeding, a larva has sucked blood for as long as twenty-five minutes, peristaltic waves being clearly visible in the gut the while. Usually ten to fifteen minutes' feed was sufficient.

The imaginal stage is attained about eleven to twelve days after hatching, sexual maturity about four days later. Copulation is intermittent, but frequent. In several cases it occurred shortly after feeding, particularly when the insects fed greedily, so that unchanged fresh blood occasionally passed from their bodies after the semi-solid digested blood *débris* had ceased to be voided. Egg-laying at the rate of four or five per day occurs during the rest of the life of the female, who is longer-lived than the male. Warburton found that the adult life of a male was about three weeks, that of a female four weeks. In my own experiments similar results were obtained, but I also found that the length of life was sometimes about a week less, in each sex.

The mode of feeding of adult *P. vestimenti* is of interest. After settling down on the hand, often clinging to the scrap of cloth on which they usually rest, a fairly sharp stab is made, and immediately the blood begins to flow into the alimentary canal, which becomes bright red. As feeding proceeds, the louse gradually raises its abdomen, until it is almost vertical in extreme cases. As fresh blood passes into the gut, defaecation occurs, much excrement being produced. If an attempt be made to remove a louse before it has finished its feed, the pull of the ring of hooks near the lower lip can be felt. Lice fed in a somewhat restricted area showed no hesitation in sucking blood at spots fouled by themselves or their neighbours. Adult lice would feed for twenty to thirty minutes. If feeding were neglected, the lice died in about three days. I found it necessary to feed them at least twice daily, though I have succeeded in keeping two females alive for three weeks when fed only once a day. Larvae perish if not fed within thirty-six hours of hatching, and even then there is great loss during the larval stage.

Lice are also very sensitive to changes of temperature. Body heat seems necessary for them, though eggs can withstand great extremes of temperature.

Death of *P. vestimenti* appears to occur very suddenly. I found that a fair number of those adults that died did so within a short time after a meal, their alimentary canals containing much unchanged blood.

Regarding the specific name of the body-louse there is much uncertainty. Neumann, in a recent paper (July, 1911), suggests that *P. vestimenti* is a sub-species of *P. capitis*, and would then be called *P. capitis vestimenti*. However, a discussion of such a difficult matter of nomenclature is quite outside the scope of this paper.

SUMMARY AND CONCLUSIONS

1. *Herpetomonas pediculi* is a parasite of the body-louse, *Pediculus vestimenti*. The parasite appears to be confined to the alimentary tract and faeces of its host (adult and larva), one phase of it having been recovered from the dejecta. The parasite is spread from louse to louse by the contaminative method, cysts of the parasite being swallowed by the insect. The whole life-cycle has been followed in the living material.

2. Movements of the flagellate are very rapid and somewhat spasmodic, and are easily accomplished by the aid of the flagellum. Rotatory motion and movements of flexion occur.

3. The parasite exhibits three well-marked developmental phases, united by a continuous series of intermediate forms:— (i) the pre-flagellate, which produces a flagellum and elongates (figs. 1-7), and becomes (ii) the flagellate (figs. 8-18), which, after a growing and multiplicative phase by longitudinal fission (figs. 19-21), forms (iii) the resting, 'encysted' post-flagellate form, adapted for extra-corporeal life (figs. 22-29).

4. Pre-flagellate stages, best found in the oesophagus and proventriculus of the louse, or in the larva, strongly resemble Leishman-Donovan bodies. They are 6μ to 7μ long and 4μ to 5μ broad. The nucleus and blepharoplast are well defined. A chromatophile area, from which the flagellum develops, is present.

5. The flagellate forms occur chiefly in the mid-gut. The body-length is from 11μ to 26μ in those I have examined. The cytoplasm is finely alveolar. The nucleus is round or oval, and the blepharoplast stains deeply. Aggregation rosettes of flagellates of various ages and sizes are occasionally found (fig. 13).

6. Post-flagellate forms are oval, usually provided with a 'skin-tight' cyst. The blepharoplast seems smaller than that of the flagellate or pre-flagellate. This stage is best observed in the rectum of the louse, and can be recovered from the faeces.

Radially striated, thick-walled cysts occur very rarely (fig. 29).

7. My experiments show that *H. pediculi* is not a stage of a vertebrate trypanosome, for I have fed the infected lice from the time of hatching to the time of death on my own body, and have made detailed examinations of my own blood by smears, thick films, and by cultures, as well as by sub-inoculations into white rats, none of which has ever given indication of trypanosomes during three years of experiments. Animals inoculated with *H. pediculi* from the gut of lice have also shown no parasites.

8. *H. pediculi* is a parasite of the louse, *Pediculus vestimenti*, and shows no connection with any vertebrate trypanosome. Also, it is not connected with *Leishmania*.

REFERENCES

Further references will be found in some of the memoirs cited.

Léger, L. (1902). 'Sur la structure et le mode de multiplication des Flagellés du genre *Herpetomonas*, Kent,' C.R. Acad. Sci., Paris, CXXXIV, pp. 781-784. 7 figs.

Neumann, L. G. (1911). 'Notes sur les Pédiculidés, II,' Archives de Parasitologie. XIV, pp. 401-414. (*Pediculus*, pp. 410-413).

Porter, Annie (1909). 'The Life-cycle of *Herpetomonas jaculum* (Léger), Parasitic in the Alimentary Tract of *Nepa cinerea*', Parasitology, II, pp. 367-391. 1 plate.

Porter, Annie (1911). 'The Structure and Life-history of *Crithidia pulicis*, n. sp., Parasitic in the Alimentary Tract of the Human Flea, *Pulex irritans*,' Parasitology. IV, pp. 237-254. 1 plate.

Saville-Kent, W. (1881). 'A Manual of the Infusoria.' 3 vols. London. (*Herpetomonas*, p. 245.)

Warburton, C. (1909). 'A Preliminary Investigation on Flock as a Possible Distributor of Vermin, and on the Life-history of the Body Louse (*Pediculus vestimenti*).' Reports to Local Government Board on Public Health and Medical Matters (New Series, No. 2). 5 pp. London, H.M. Stationery Office.

EXPLANATION OF PLATE I

All figures outlined with Abbé-Zeiss camera lucida, after wet fixation with osmic vapour and absolute alcohol and staining with modified Romanowsky solution; 2 mm. apochromatic objective (Zeiss) and compensating oculars 8 and 12 used. Magnification 1500 diameters.

Figs. 1 to 7 represent pre-flagellate and intermediate forms of *H. pediculi* from the digestive tract of the larva or the fore-gut (oesophagus and proventriculus or anterior lobe of the stomach) of the adult *Pediculus vestimenti.*

In fig. 1 note the chromatophile or pink-staining finely granular area from which the flagellum arises.

In figs. 2, 3, 4, 6, 7 note the gradual lengthening of the flagellum, and elongation of the body.

Fig. 5 represents a late pre-flagellate organism dividing.

Figs. 8 to 18 show flagellate parasites from the stomach and anterior part of the intestine (or mid-gut) of the adult louse.

Fig. 8. Young flagellate.

Fig. 10. Flagellate showing body twisted or folded over about the middle of its length.

Fig. 13 represents an aggregation rosette, or cluster of flagellates, of different sizes and ages.

Figs. 14, 15, 16 show parasites whose bodies are thrown into characteristic undulations. Note the chromatoid granules shown in fig. 15.

Figs. 17, 18 represent stout flagellates from the anterior part of the intestine. The latter figure shows the stoutest parasite seen during the research. The parasite contained chromatoid granules.

Figs. 19 to 21 represent dividing forms.

Figs. 22 to 25 show stages of the parasite leading to post-flagellates and cysts (figs. 26-29), as seen in the hinder part of the intestine, including the rectum and the faeces.

In figs. 22-25 note the gradual shortening and absorption of the flagellum, and the contraction and rounding of the body. In fig. 25 only the rhizoplastic part of the flagellum remains. Chromatoid granules occur in these parasites.

Figs. 27 to 29 represent truly encysted forms, as found at the extreme posterior end of the gut, or voided in the faeces with semi-solid, black blood remains.

In figs. 27, 28 the cyst-wall is thin and varnish-like, and closely apposed to the parasite. It stains pink after Giemsa.

Fig. 29 represents a gelatinous, thick-walled cyst with striations. Such cysts were very rare.

NOTE

Dr. Stannus wishes to make the following acknowledgments regarding the paper on "A Case of Human Trypanosomiasis in Nyasaland, with a note on the Pathogenic Agent," by Drs. H. S. Stannus and Warrington Yorke. (Annals of Tropical Medicine and Parasitology, Vol. V, No. 3 (1911), page 443.)

Through an oversight, failure was made to acknowledge our indebtedness to Dr. William Murray of Mvera, and to Dr. Meredith Sanderson in charge of the Sleeping Sickness Investigation, for clinical notes and record of temperature of the case therein recorded.